

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

APPLICANT:	MESSIER	}	EXAMINER:	HORLICK, K.R.
SERIAL NO.:	10/079,042		ART UNIT:	1637
FILED:	FEBRUARY 19, 2002		CONF NO.:	5992
TITLE:	EG307 POLYNUCLEOTIDES AND USES THEREOF			

**DECLARATION OF WALTER MESSIER  
UNDER 37 CFR §1.132**

Dear Sir:

1. I, Walter Messier, Ph.D., am the named inventor of the above-referenced application and the Chief Technology Officer of assignee. I received my MS from the State University of New York at New Paltz, and my Ph.D. from the University of Albany (State University of New York). I have published in such prestigious scientific journals as Nature and Current Biology. I am recognized as an authority on the use and interpretation of Ka/Ks algorithms and am of skill in the art in the area of research on the detection of molecular-level positive selection in plants.

2. Described below is an additional experiment which was carried out and the data analyzed under my direction and authority, testing whether there was positive association of EG307 with yield.

3. An association analysis was carried out to determine whether EG307 and EG1117 genes were associated with yield in rice. 104 well-characterized rice strains were analyzed for EG307 and EG1117 alleles. Genotyping was performed by means of DNA sequencing. DNA was prepared using standard methods. Association was analyzed using a standard general linear model (GLM) and statistical analysis incorporated in software packages such as TASSEL. Table 1 lists statistically significant  $R^2$  values for several traits.

**Table 1. Association Analysis in Rice for EG1117 and EG307**

TRAIT	$R^2$ VALUE
GRAIN YIELD (LBS)	26%
WHOLE MILL	31%
SEED WEIGHT	26%
LODGING	31%
TOTAL MILL	18%
CHALK	20%
ANTHESIS	13%
AMYLOSE	10%

4. As can be seen from this association data, there is a very strong correlation  $R^2$  value with grain yield (lbs), whole mill, and seed weight, all measures of yield.  $R^2$  values indicate the amount of variation in a particular trait that can be explained by the genetic locus being analyzed.

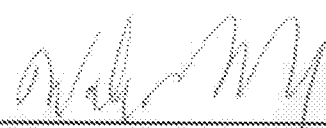
5. As discussed in the Specification, EG307 and another gene, EG1117, described in related applications, map to the same Clemson BAC contig, 58. EG1117 is about 3 cM upstream of EG307. The RGP database puts these two genes 2 cM apart on chromosome 3. Thus, these genes are located close together on chromosome 3, and thus cosegregate in this analysis. Accordingly, the  $R^2$  values for both EG1117 and EG307 are the same.

6. I conclude from this data that the gene EG307 in rice is strongly associated with yield in rice and that the "domesticated" EG307 allele found in many commercial "domesticated" rice strains increases yield in rice over the ancestral, non domesticated rice strains.

7. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Date: 3/14/07

  
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Walter Messier, Ph.D, Chief Technology Officer  
Evolutionary Genomics, LLC